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TITLE: PRODUCTION OF ANTIGENS AND ANTIBODIES FOR DIAGNOSIS OF ARBOVIRUS DISEASES

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Rabbits were immunized successfully intravenously with 9 arboviral immunogens grown in RK-13 rabbit cells. Testing of the resulting ammonium sulfate precipitated IgG by ELISA showed generally excellent titers. The testing is continuing.

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SUMMARY

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Robert E. Shope May 20, 1994

PI Signature Date

TABLE OF CONTENTS

BODY OF REPORT

1. **Production of mouse brain sucrose-acetone extracted antigens.**

Twenty-five antigens were prepared during the project year as listed here:

Antigen	Strain	Passage	Number of lots	Volume to date (ml)
Bandia	RV611	sm8	1	217
Belterra	BeAn356637	sm4/5	5	239
Bhanja	IG690	sm20	3	237
Bunyamwera	prototype	sm48	1	269
Chagres	JW10	sm10V1sm1	2	97
Chandipura	I653514	sm4	5	310
Dugbe	IbAr1792	sm12	2	105
Germiston	SaAr1050	sm17/19/20	1	473
Hazara	JC280	sm10	9	266
Hughes	DT	sm20/21	7	280
Ilesha	prototype	sm13/14/15	6	213
Ilheus	Laemmert	sm40	3	305
Mayaro	TR15537	sm6/7	2	229
Maguari	BeAr7272	sm9	6	223
O'nyong-nyong	Gulu MP30	sm12/13	9	375
Qalyub	EgAr370	sm4	2	338
Quaranfil	Ar1095	sm20	4	279
Semliki Forest	Smithburn	sm13/14	4	182
Salehabad	I81	sm16V2	1	23
S.f. Sicilian	Sabin	sm37V2	2	37
Snowshoe hare	RML	sm16	5	306
Toscana	ISS Ph1-3	V1sm2	7	314
VS-Indiana	Indiana Lab	sm9	5	271
VS-New Jersey	Hazelhurst	CE18V4sm1	4	364
Zika	prototype	sm151	11	501

Additionally, 8 viruses were passaged in baby mice awaiting preparation of antigen lots. These were Bussuquara(4 lots), Cocal (3 lots), Jamestown Canyon (3 lots), Oriboca (6), Piry (2), Ross River (1), Sindbis (1), and West Nile (1).

2. Production of antibody to arboviruses in rabbits.

Rabbits were immunized to Semliki Forest, Cocal, Chandipura, Bussuquara, Mucambo, Quaranfil, Jamestown Canyon, snowshoe hare, and Piry during the contract period. As before, the rabbits responded well when boosted two or more months after the primary series of intravenous inoculations of RK-13 grown viruses. Results of ELISA with ammonium sulfate concentrates were:

Virus	Optimal titer in ELISA	Volume (ml)
Hazara	1:500	15
	1:64000	42
Ilheus	1:64000	18
Bwamba	1:500	15
Quaranfil	1:4000	85
	1:8000	95
Chandipura	1:500	27
	1:800	47
	1:4000	43
	1:32000	87
Bussuquara	1:400	15
	1:200	28
	1:2000	40
	1:16000	34
	1:32000	10

Antigens for the above tests were used at 1:10 except for Bussuquara which was used at 1:25.

DISCUSSION AND CONCLUSIONS

The problem of technical personnel after the death from cancer of the project's technician, was partially solved. An Associate in Research was recruited, trained and worked well during 6 months of this contract period. He was lost to the project in January when he entered a Ph.D. program. The back-log of antigen production was lessened significantly. A no-cost extension was negotiated and another Associate in Research has been recruited to start in the next contract period. Emphasis will be on completing the sucrose-acetone extraction of antigens and further ammonium sulfate purification, followed by ELISA, of the rabbit IgG.